The University Of Sheffield.

Bioinformatics for high-throughput data

BMS353

Today’s Outline

Part A : Introduction on high-throughput data
Experimental design
Platforms for gene expression data

Break – 10 min (questions if any)

Part B : Exponential and logs
Low level analysis

- Gene Expression Estimation
- Normalisation

Class activity: Research on burning questions.

Today we will be learning:

- Characteristics of high-throughput data and how it is produced
- What we intend for OMICS
- How we define high-throughput data
- To define principles of experimental design and pipelines
- Methods for gene expression quantification
- To estimate gene expression levels from data and difference in methods applied
- To normalise the data and what it means

High-throughput data

High-throughput data is a large amount of data collected using automated methods and non-conventional technologies. This is to be able to perform a large number of experiments at the same time to monitor a behavior of a system.

High-throughput data is produced in all field where quantitative science is applied.
For example for robotics and automated system, in ecology to study and monitor populations, in chemistry for compound screening and obviously in biology.

High throughput *cell biology* is the use of automation equipment with classical cell biology techniques. This is to address biological questions that are otherwise unattainable using conventional methods.

High-throughput biology has created a new field of biology called *OMICS.* It is a research filed that interface between large scale biology (genome, proteome, transcriptome), technology and computational methods.

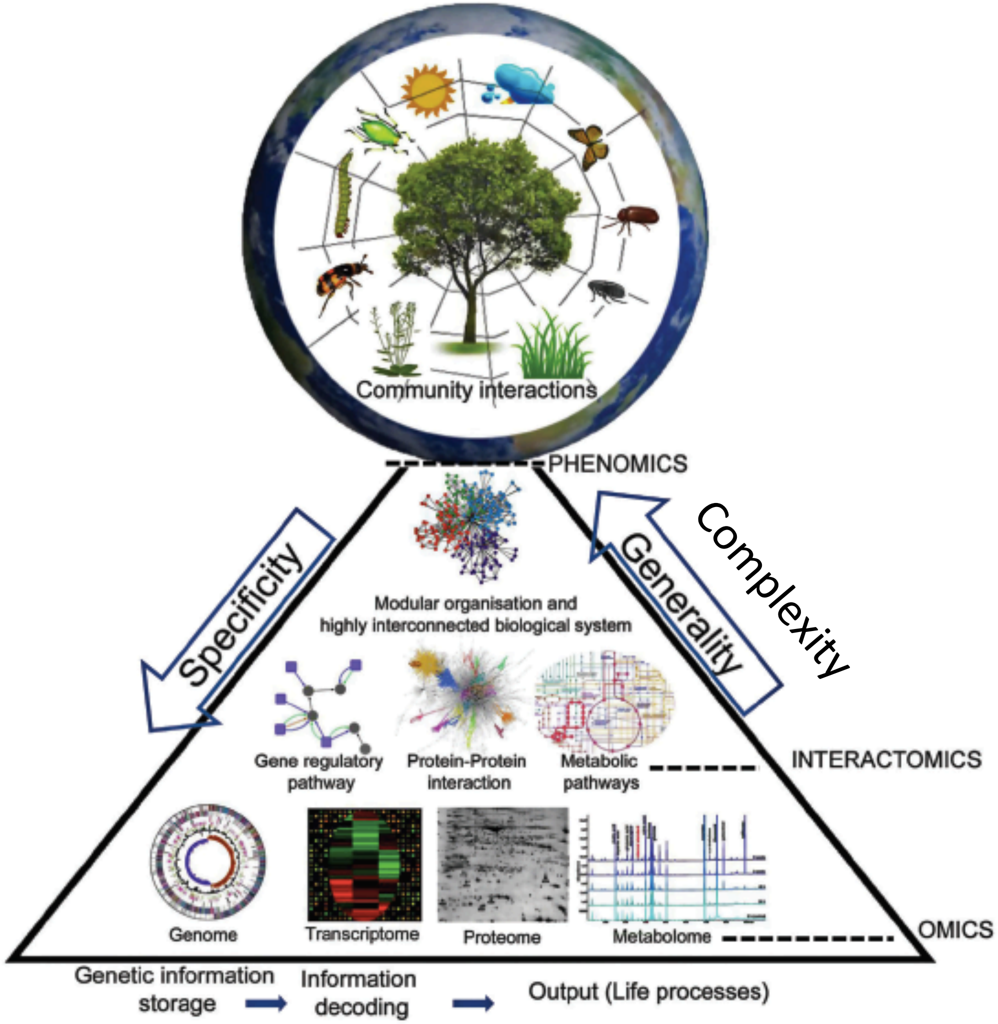
Characteristics of high-throughput data

What are the characteristics of high-throughput data?

- Large in size
- Prone to many false positives (low specificity)
- Capture biological Noise
- Accurate
- Technically noisy

The University Of Sheffield.

Omics and its hierarchy



To microscopic to macroscopic

The highly interconnected hierarchical organization and functional complexity need a sophisticated integrated systems approach.

Journal of Experimental Botany, Vol. 66, No. 2 pp. 479–493, 2015

Why do we need mathematics to understand biology?

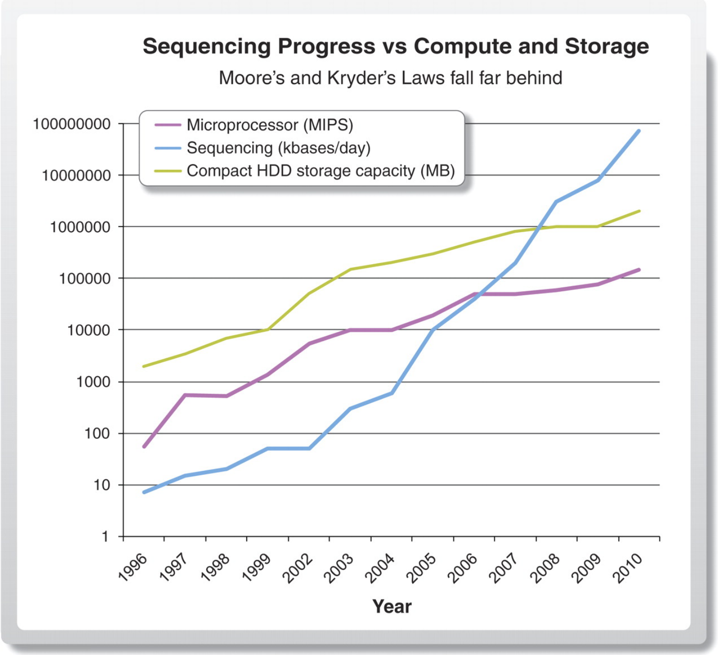
Quantification of genome wide gene expression Gene networks and target predictions Protein-Protein Interaction Networks	**Biological systems**
Alignment tools Mining of large data Accessing large data from repositories	**Large Data**
Handling complexity High dimensions Patterns in the data	**Patterns and structures**
Quantification of Uncertainty: Be able to predict what we cannot measure and have a theoretical way of quantify accuracy	**Modeling**

Computational Biology --- Bioinformatics

Genomic Data: the truth about it

In February 2001 scientists published the first drafts of the Human Genome. The dawn of the genomic era.

Challenges in genomics derive mainly from the *informatics* that is required to **analyse, summarised** and **interpret** the vast amount of raw (sequencing and not) data that is available.



S D Kahn, Science 2011;331:728-729

Moore's law: The number of transistors that can be placed inexpensively on an integrated circuit doubles approximately every two years

Current information technology allows to perform standard analysis of raw output from sequencing in average time of 3 days on a cluster

Value and definition of raw data?
How can we analyse this data effectively?

Tools we need

- Optimal Experimental Design – minimise the noise in the measurement
- Mathematical Models – define rules (functions) to describe processes
- Statistical tools – quantify accuracy in prediction and sensitivity in estimation
- Computational Skills – handling large amount of data in automated way
- Visualisation tools – identify patterns in the data

The role of experimental design

Importance of defining your research questions, keeping in mind limitation and effective use of the data

We need to limit the uncertainty of the "unknown" by define very clear questions. This helps to :

- reduce variability in the data.
- reducing the complexity of the data by focusing the search of information on the questions you have in mind.

A proper experimental design **MUST** reflect :

- the biological questions that you are asking,
- the protocols optimised to minimise the variations in the data
- identify the limitations of the data collected.

RNA sample preparation for high-throughput assays

Experimental design:

your RNA collection needs to reflect the biological questions you are asking

Optimise protocols:

minimise technical errors

minimise batch effects

clean and pure sample to avoid contamination

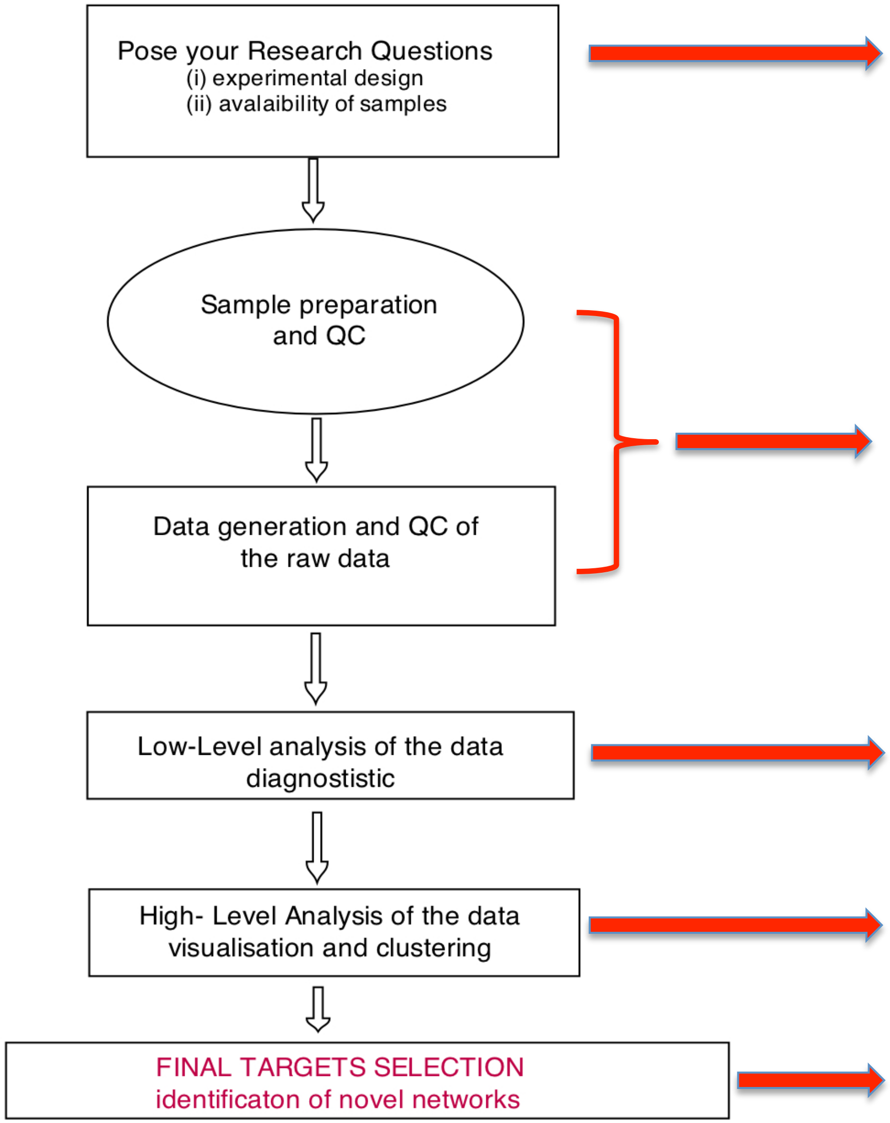
Estimate the correct quantities:

samples for validation

optimisation of the protocols – avoid saturation or low quantification

Technical and biological replicates:

ensure you have SOP in place


Pose your Research Questions
(i) experimental design
(ii) avalaibility of samples
Sample preparation and QC
Data generation and QC of the raw data
Low-Level analysis of the data diagnostistic
High- Level Analysis of the data visualisation and clustering
FINAL TARGETS SELECTION
identificaton of novel networks

1. Importance of defining your research questions, keeping in mind limitations and effective use of the data

2. Consistency in sample preparation, optimisation of the samples, extensive QC of the data. LOOK at the data generated and QC before processing

3. Choose the correct model to analyse your data, define appropriate parameters (RNA-Seq analysis) to get the maximum information out of your data

4. Use the best tool to visualise your data, to discriminate, cluster and rank your significant targets

5. Using of pathway analysis for defining novel hypothesis that can be investigated with "specific tools" , mathematical and experimental

Pipelines

All the variation and to ensure that the analysis of the data is as reproducible as the experimental collection of samples generate the need to define pipelines for the analysis of the data

PIPELINES: Reproducible and robust protocols for numerical experimentations. In case of biological data they are tailored to the system/organism under study.

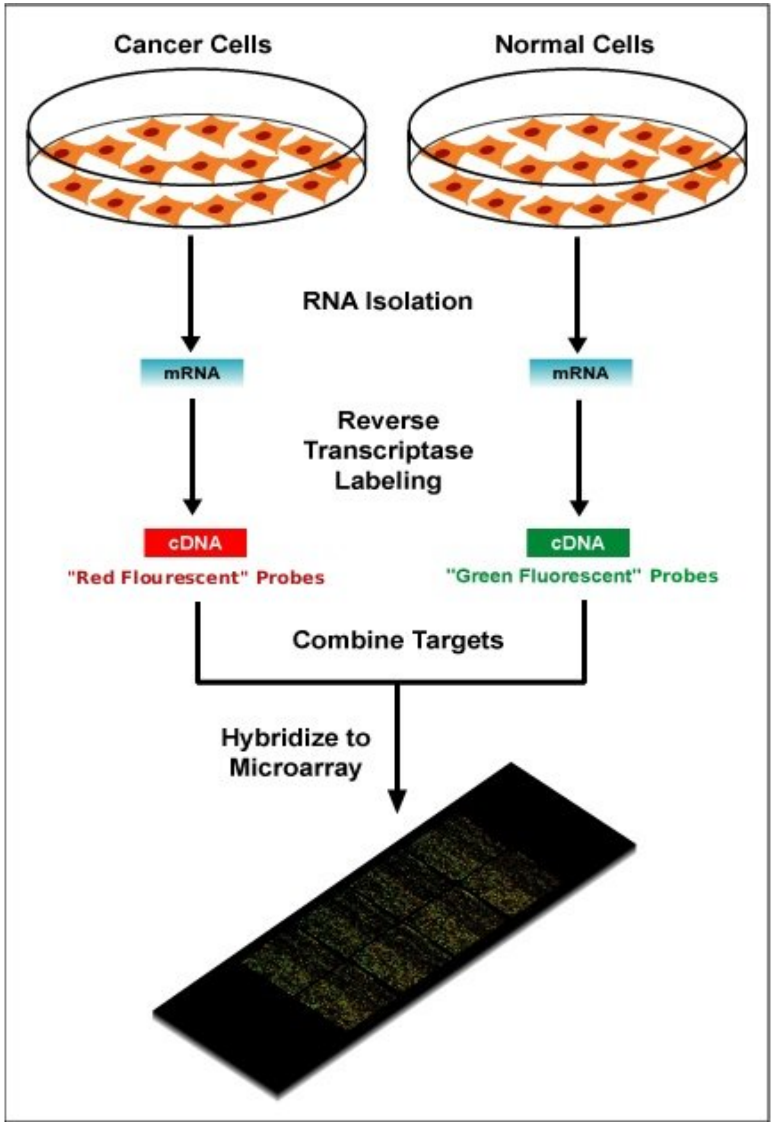
HOW DO WE GET THEM?

Gene expression estimation

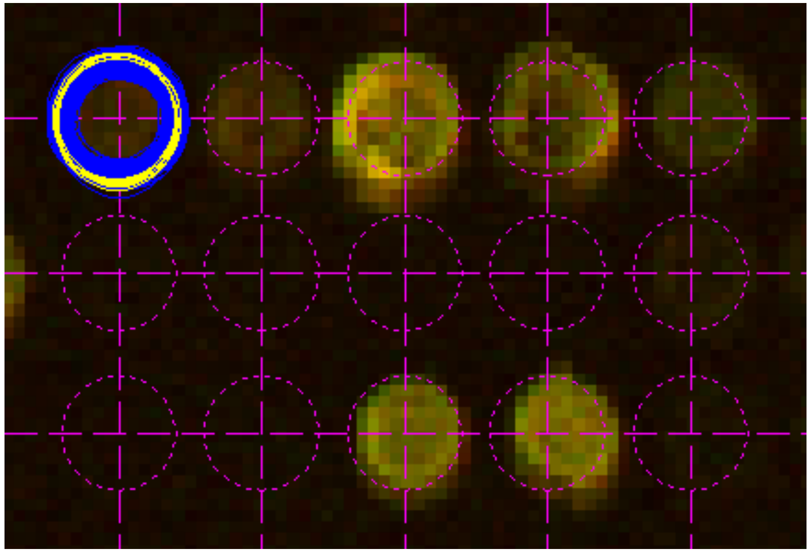
Interpret and analyse the data by first understanding where the data is coming from

Different platforms that generate gene expression:

- Two or One color spotted cDNA arrays
- Affymetrix - new Human/Mouse whole transcriptome arrays
- Illumina Arrays
- Reads – RNA-Seq and other NGS assays



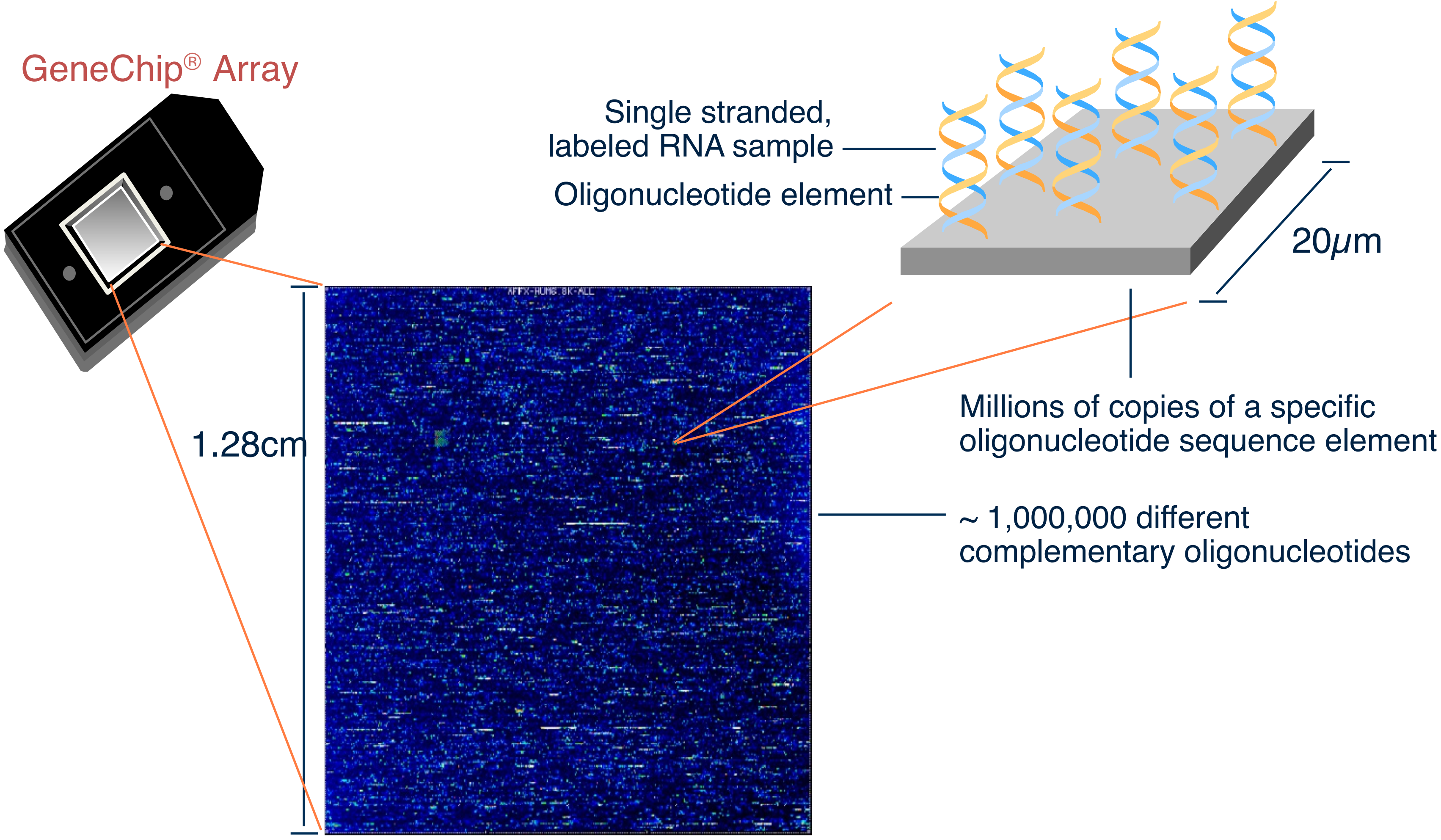
Wikipedia – DNA microarrays



How do I quantify the gene expression?

Image processing noise
Sensitivity

GeneChip® Array



Single stranded, labeled RNA sample

Oligonucleotide element

20μm

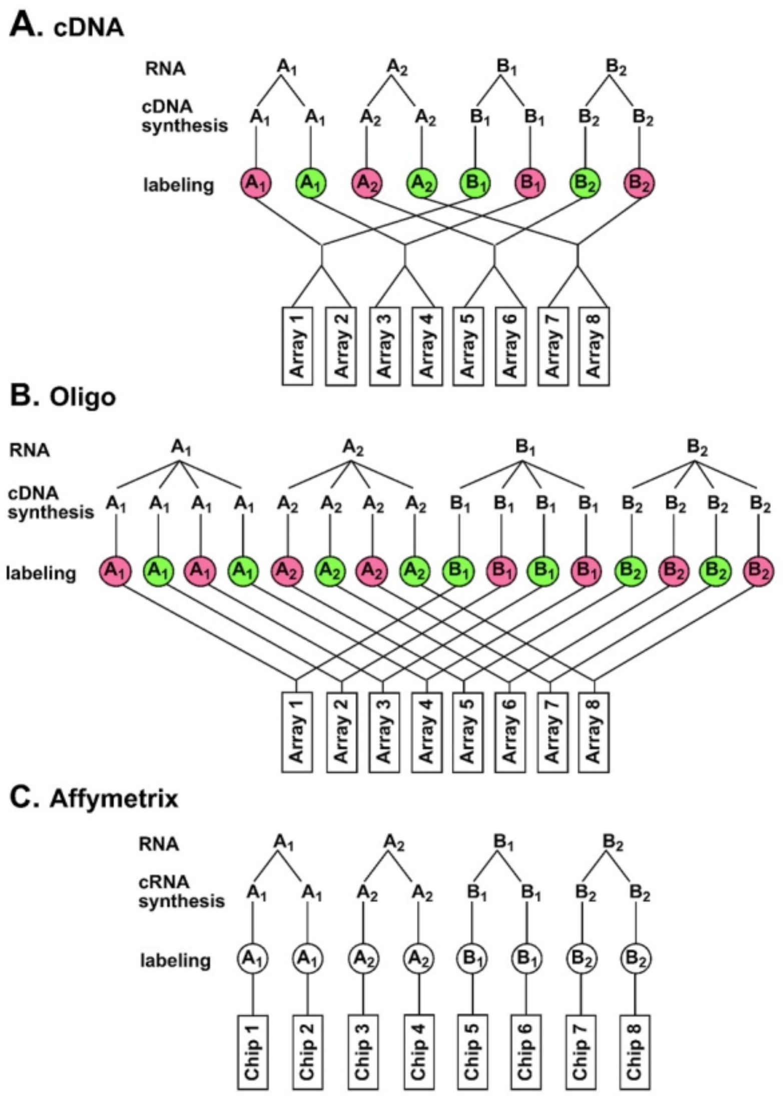
1.28cm

Millions of copies of a specific oligonucleotide sequence element

~ 1,000,000 different complementary oligonucleotides

Image of Hybridised Array

Example of experimental design



J Biomol Tech. 2004 December; 15(4): 276–284.

RELATIVE EXPRESSION

- Relative expression
- Important to choose the reference
- Important to choose the experimental design

ABSOLUTE EXPRESSION

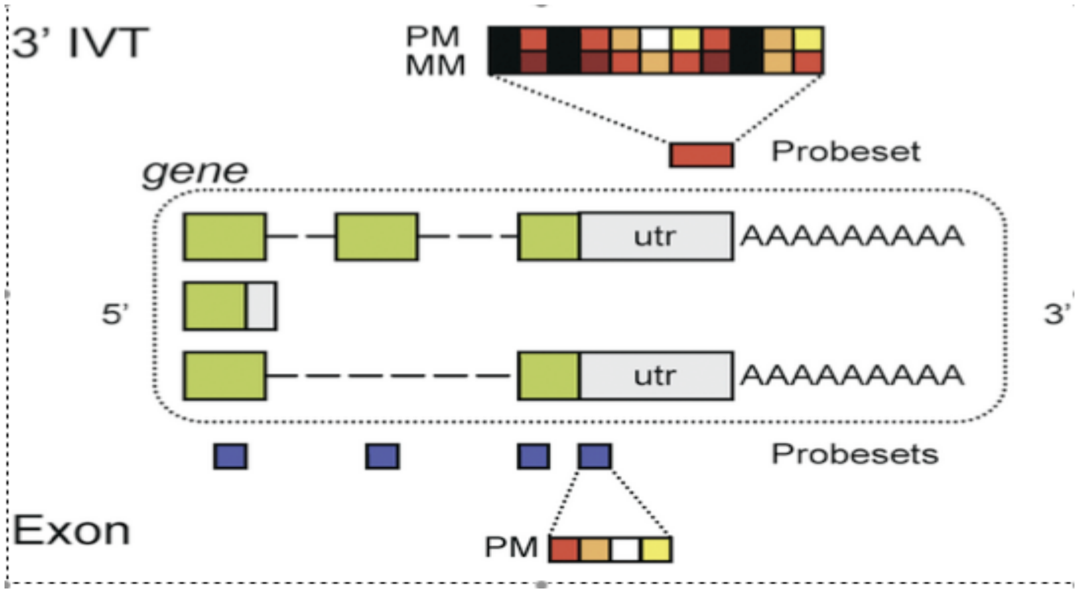
- Estimation of Absolute expression
- No reference
- Not specific to the question you are asking
- Important the design – data analysis

Example: HG_U133 Plus v2 Affymetrix geneChip

The sequences from which these probe sets were derived were selected from GenBank®, dbEST, and RefSeq.

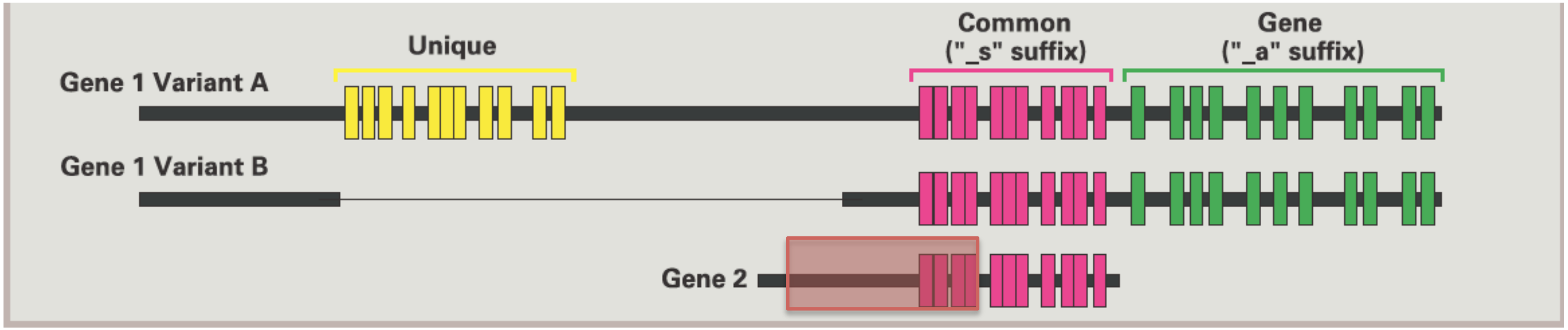
A single array contains with more than 54,000 probe sets representing approximately 38,500 genes (estimated by UniGene coverage).

70 percent of the probe sets represent subcluster assemblies containing one or more non- EST sequences. Of the 16,737 EST-based probe sets, approximately 9,000 probe sets can now be associated with an mRNA or other non-EST sequence.



Now with new arrays HJAY…..

Probe Set Notation



("_x" suffix)

_at : probe sets are predicted to perfectly match only a single transcript

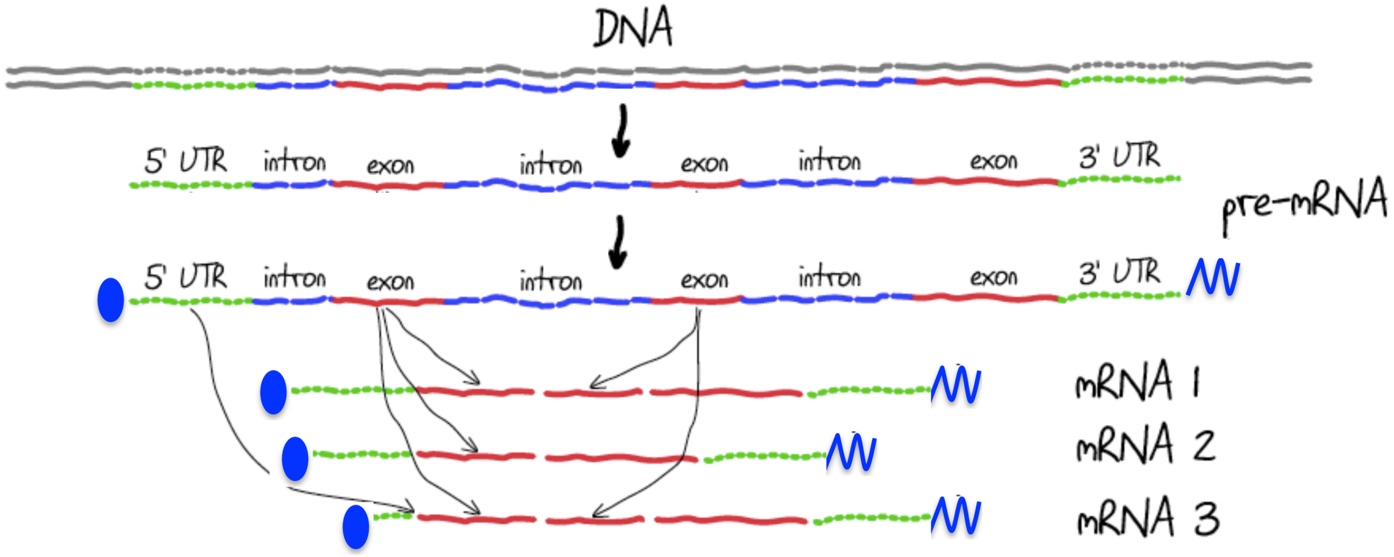
_s_at : are predicted to perfectly match multiple transcripts, which may be from different genes

a_at : all probes in the probe set hit alternate transcript of the same gene

_x_at : probe sets will contain some probes that are identical or highly similar to other sequences from different gene.

Hybridize uniformly across probe pairs to the intended target

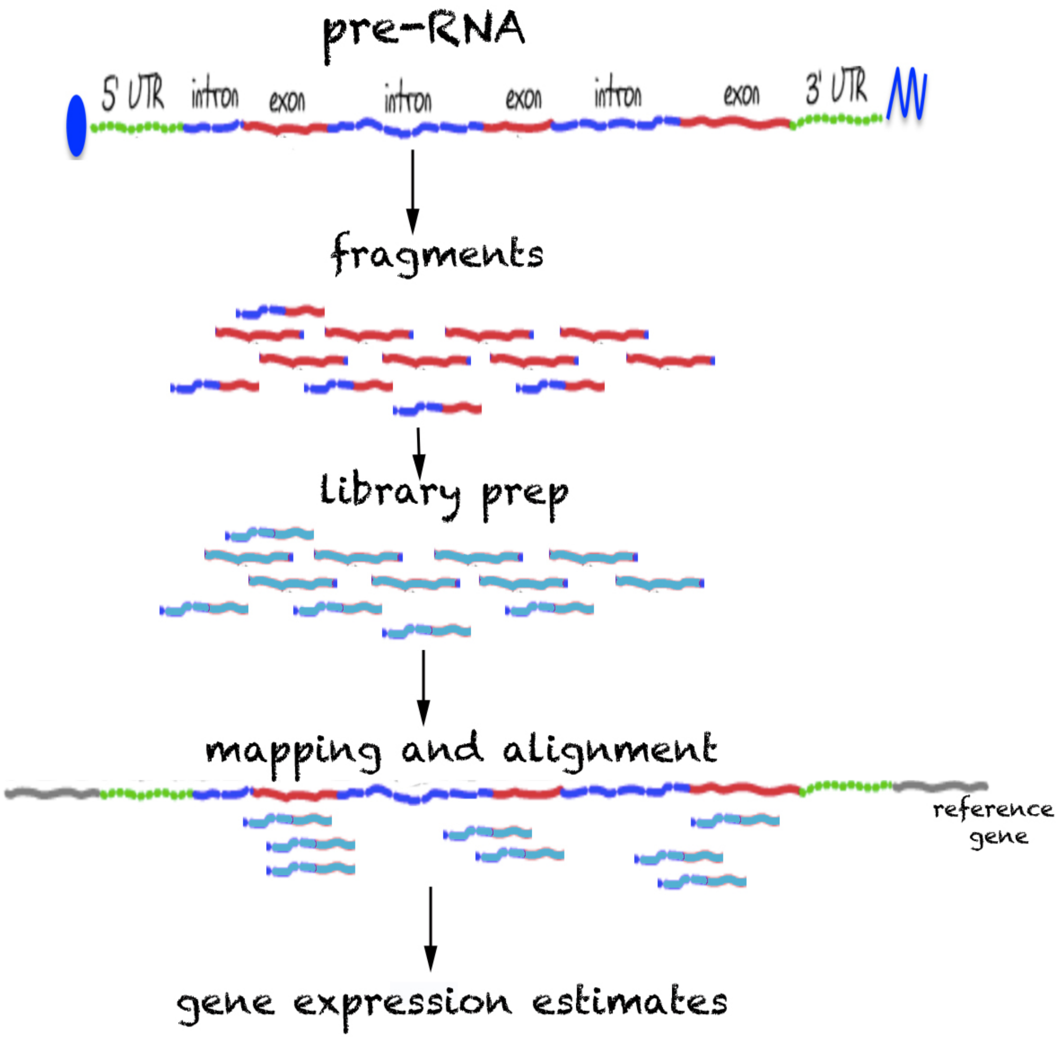
Gene expression quantification using RNA-Seq



High-throughput sequencing of cDNA:

- Shared exons
- Biological variance of fragments
- Splicing variations

Example of RNA-Seq workflow



Illumina sequencing – HiSeq

Illumina HiSeq sequencer

What are we able to detect?

- Mapped read count proportional to abundance of fragments
- Abundance of fragments ≈ (gene expression) x (length)
- Problems which length? which transcripts?
- Other difficulties: mismatches, varying quality of reads, non-uniform read distribution

Part B

What's in the GeneChip data?

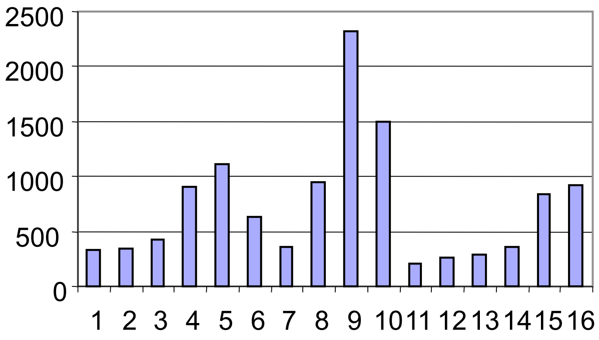
1. Summarise to a single expression level the probe intensities for each probe set

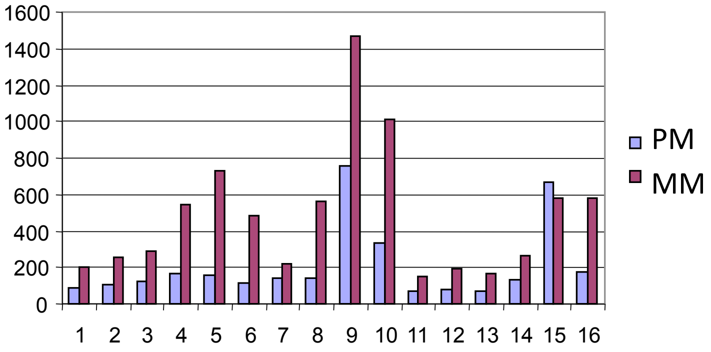
2. Estimate the variations introduced by
   background effect
   probe affinity effect

3. Some PM/MM pairs are more reliable than others

4. The signal needs to be scaled before comparing data from different arrays

How we define a measure that best represent the absolute expression level of each gene on the chip?





The approaches

Use **single point statistics**

make use of the information we have to define values that estimate gene expression

MAS 5.
RMA – GCRMA
PLIER

Use a **probabilistic approach (in Week 4)**

make use of the observed data to estimate function that have generated that data

Estimates of gene expression will be the most probable value that summarises the probe set

PUMA

Data transformation

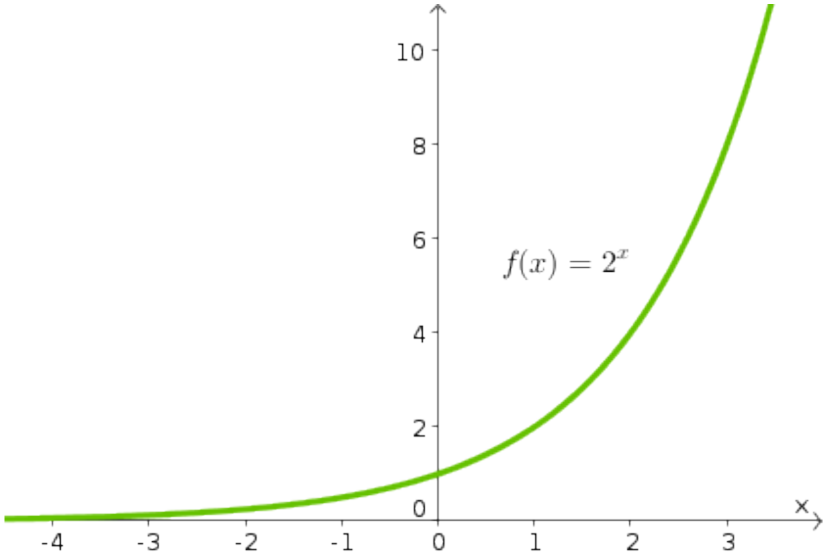
Data transformation converts a set of data values from the format of its original source into a new format that better suits the destination system where the data is then manipulated/used/ mined etc...

In statistics, data transformation refers to the modification of every point in a data set by a mathematical function.

When applying transformations, the measurement scale of the variable is modified.

Data transformation is most often used to change data to the appropriate form for a particular statistical test or method.

Data Transformation: Exponential functions



$$1 + x + x^2 + \ldots + x^n$$

$$x^n * x^m = x^{n-m}$$

$$x^n \,/\, x^m = x^{n-m}$$

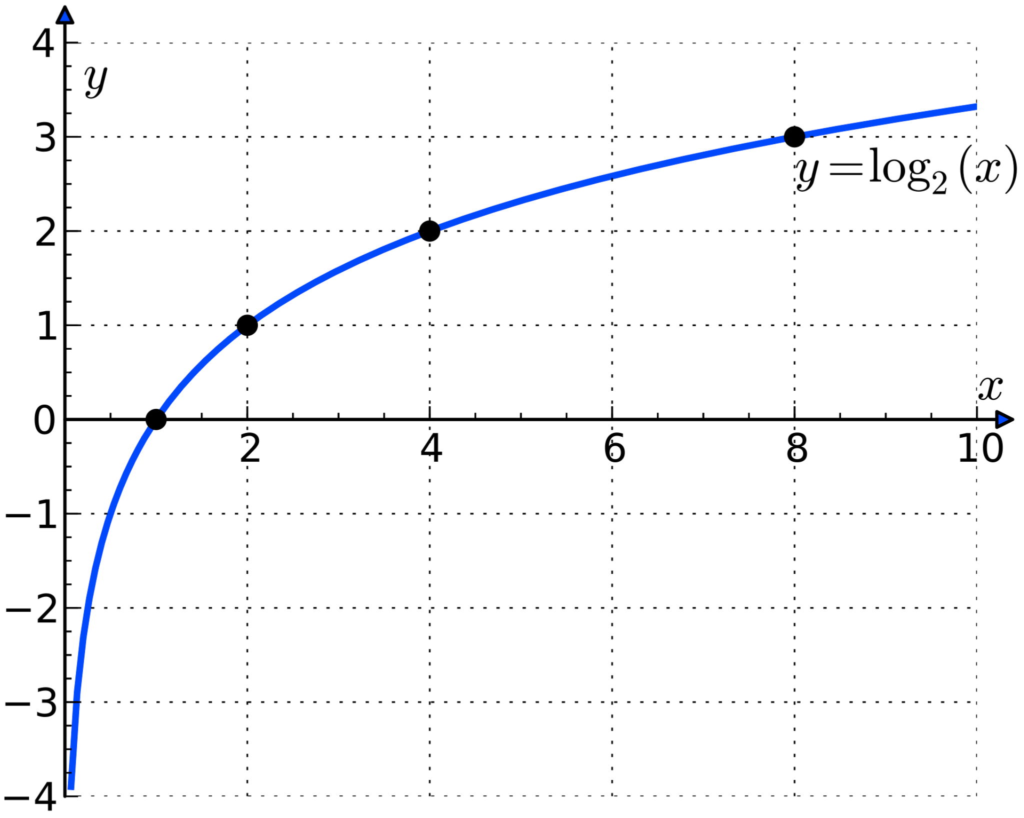
$$y = f(x) = e^x = \exp(x)$$

The number **e** is the Euler's number, an irrational number
The first few digits are:
2.7182818284590452353602874713527 (and more ...)

Gaussian Function.

$$y = f(x,\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi}}\exp(-\frac{(x-\mu)^2}{2\sigma^2})$$

Data Transformation: Logarithmic functions



$log_b(xy) = log_b x + log_b y$

$log_b(x/y) = log_b x - log_b y$

$log_b(xn) = n\ log_b x$

$log_b x = log_a x \,/\, log_a b$

y = log$_b$x if and only if by = x,
where x > 0, b > 0, and b ≠1.

Microarray Suite (MAS5.0)

$$Signal \sim TukeyBiweight(log_2(PM_j - IM_j))$$

Correction for global background.- based on 16 sectors on each array

Ideal mismatch (IM) intensity calculated from MM value and subtracted from PM.
  - if MM < PM then IM = MM
  - if MM > PM then IM = PM – correction value

- Signal = Smoothed average over PM/MM pairs representing a gene
- Signal is always positive: Absent - Present Call

MAS5: p-value and calls

- First calculate discriminant for each probe pair:

  $$R=(PM-MM)/(PM+MM)$$

- Wilcoxon one sided ranked test used to compare R vs tau value and determine p-value
- Present/Marginal/Absent calls are thresholded from p=value above and
  - **Present** =< alpha1
  - alpha1 < **Marginal** < alpha2
  - Alpha2 <= **Absent**
- Default: alpha1=0.04, alpha2=0.06, tau=0.015

Not very precise, accurate only when many replicates are available.
Dependent strongly on MM,
Uses linear scaling normalisation

Robust Multi-array Average (RMA)

$$Signal \sim Tukey\ (log_2(PM_j - bkgd_j))$$

- Subtract background for each array from PM
- Intensity- dependent normalisation of PM-Bkgd
- Log transform
- Robust multichip analysis of all PM reporters in the set using Tukey median polishing procedure
- Quantile normalisation :Fit all the chips to the same distribution. Scale the chips so that they have the same mean.

Normalisation

Why do we need to normalise the data?

1. we want to compare across chips
2. we need to ensure that all the data is equally compared across baseline within the chip

Most methods will have normalisation step incorporated, some other will need to perform it after gene expression estimation

Scaling – Mean and Median
Quantile

Loess (not relevant for affymetrix data and sequencing data)

Normalisation: scaling

The assumption that mapping using quantiles or scaling is reasonable is based on the assumption that "most genes don't change", and quantiles use this more extensively than scaling.

If this underlying assumption is doubtful, then using the above methods is not advisable.

Simply linearly scale the gene expression so that the overall mean / median are the same.

The median is more scale-invariant, but for the most part there is little practical difference.

Normalisation: quantile

In statistics, *quantile normalization* is a technique for making two distributions identical in statistical properties. When we quantile-normalise a sample distribution to a reference distribution of the same length, we align the sample distribution to the reference so to make them the same.

Assume that the distributions of probe intensities should be completely the same across chips.

Start with *n* arrays, and *p* probes, and form a *[p,n]* matrix X.

Rank first:
Sort the columns of X, so that the entries in a given row correspond to a fixed quantile.

Then align:
Replace all entries in that row with their mean value.

- Visualise the effect: M-A plot

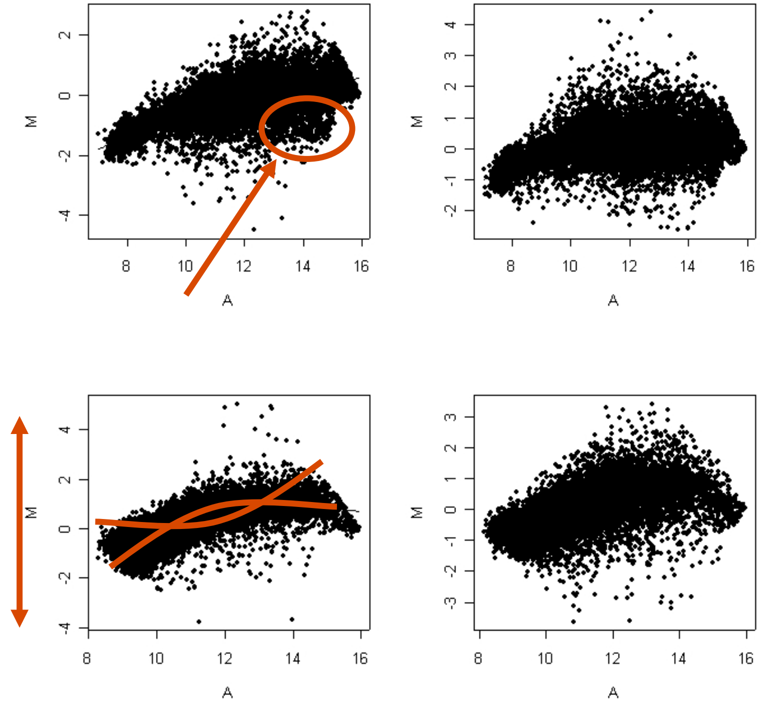
$$M = \log_2\left(\frac{R}{G}\right)$$

$$A = \log_2(RG)$$

- Correction of the intensity dependant variations:

$$\log_2(\text{ratio}) = \log_2\left(\frac{R}{G}\right) - c(A)$$

$$c(A) = \log_2 k(A)$$

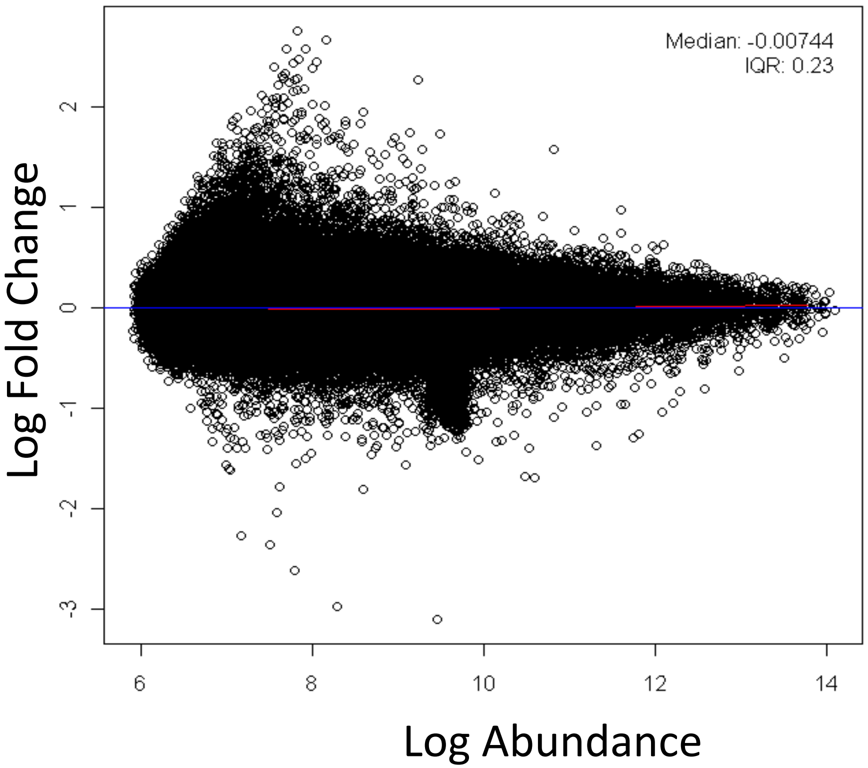


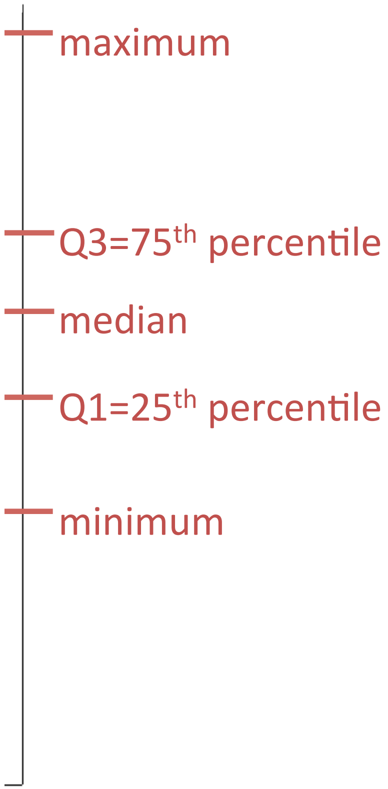
Data Visualisation

Scatter Plot

Box Plot

MA Plot





1 2 3 4 5 6 7 8 9 10 11 12

Channel

For NGS gene expression quantification….

A lot changes … but

- Estimation of gene expression
- Alternative splicing identification
- Alternative isoform detection
- Transcripts abundance

QUANTIFICATION

↓

- Normalise read counts
- Normalise reads between lanes
- Normalise reads against transcripts abundance and gene length
- Varying sequencing depth
- Other technical effects

NORMALISATION

↓

Visualise and interpret
Differential expression
Clustering

HIGH LEVEL ANALYSIS

Class activity: debate the following questions:

1. How much do I need to know about the system that I am studying?

2. How much the technologies that are available for data collection need to to sensitive for my system?

3. What is sensitivity and specificity?

4. In sequencing what is a reference genome and how I get it?

5. When the high-throughput approach is the correct approach for my research question?